Rewriting Game Theory Applied to Protein Signalling in MAPK Cascades

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Abstract. We propose the recent notion of *rewriting game theory* as a tool for studying biochemical systems. Rewriting game theory is based on a discrete and dynamic notion of Nash-style equilibria for games without structural constraints and with arbitrary payoff values. Our aim here is to show how the formalism can be used to characterise biological information as logical properties of a purely chemical model. Specifically, we address MAPK cascades through a compendium of the involved chemical reactions, with particular focus on the known signalling pathways. We also present preliminary computerised support for our methodology.

1 Introduction

The MAPK cascades are among the best studied biochemical processes, in part because they assume central positions in several species, including in humans. Evolutionary speaking, they are highly evolved and robust. Their chemical underpinning is *kinase*, i.e., the transfer of phosphate between proteins, while their biological role concerns cell growth, stress response, and others.

Game theory addresses situations with potential conflicts of interest. The core concept in non-cooperative game theory is that of Nash equilibria, prescribing compromises that satisfy all players. In evolving situations, Nash equilibria are often interpreted as good approximations of what will happen in practice.

In the life sciences, Maynard Smith has famously recast Darwinian evolution into game theory, with survival of the fittest amounting to the fact that they are the dominating species in (particular kinds of) Nash equilibria [34]. We propose to view this in the light of the central hierarchy of abstractions in the life sciences, namely chemistry $\rightsquigarrow$ biochemistry $\rightsquigarrow$ biology $\rightsquigarrow$ ecology $\rightsquigarrow$ evolution. What Maynard Smith showed relative to the hierarchy was that the *functional units* at the evolutionary level are given as Nash equilibria at the ecological level.

In this paper, we aim to apply game theory to the bottom of the hierarchy. In particular, we show how to characterise discrete and dynamic biochemical issues, like the pathways that cell signals follow, by a chemical application of a recent lightweight game-theoretic formalism called *rewriting game theory* [31]. The technology we discuss has been implemented in Mathematica and the source code is available on the homepage of the corresponding author: `http://www.`

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jaist.ac.jp/~vester/. Throughout the article, verbatim typesetting indicates that the text is lifted from an interactive Mathematica session.

1.1 Related Work

The MAPK cascade, in particular the ERK part, has been extensively analysed using mathematical tools that are stochastic, algebraic, deterministic, hybrid, etc. in nature. These have ranged from models aimed at understanding the molecular interactions of the chemical species involved in the pathways as a whole [25] and the effect that changing environmental conditions has on the kinetic behaviour of the most important contributors to the pathways [33]. Model predictions of cellular processes using various mathematical tools exist [19]. Some studies have concentrated on specific intrinsic pathway properties in order to gain a deeper understanding of the complexity of signaling systems. These include specificity [15], cross-talk [9], feedback control [2, 6, 32]; ultrasensitivity [10]; scaffolds and the complexity of formation [18]; oscillations [12] and receptor dimerisation [33], among many others.

Game theory (in the classic, probabilistic form) has been used to study the evolution of biochemical systems [27].

In [4], we apply rewriting game theory to Kauffman/Thomas-style gene-regulation analysis. The concern there is to provide a mathematical foundation for an established analytic technique that so-far has been ad hoc.

1.2 This Article

In Section 2, we discuss MAPK cascades; in Section 3, (rewriting) game-theory background; in Section 4, we introduce *cascaded proteins games*; in Section 5, we do a medium-scale application. Appendices A and B define abbreviations and *strongly connected components* and their *shrunken graphs*.

2 Signal Transduction Systems and MAPK Cascades

Cells respond to external stimuli using signalling pathways. These encompass all the biological and biochemical phenomena that start with perception of an extracellular signalling molecule (aka ligand) to the response of the cell. An elaborate system of proteins, from trans-membrane receptor proteins via cytosolic proteins to target proteins in the nucleus, enable the cell to respond to a particular signal in a specific manner. Responses include cell growth, survival, apoptosis, differentiation and proliferation [1, 26]. Intracellular proteins include kinases, phosphatases and GTP-binding proteins (GTPases). Target proteins can be ion channels, cytoskeletal and gene regulatory, just to mention a few [1].

MAPK signal transduction pathways are among the most widespread in eukaryotes [13] and are the focus of our present work. In mammalian systems, five

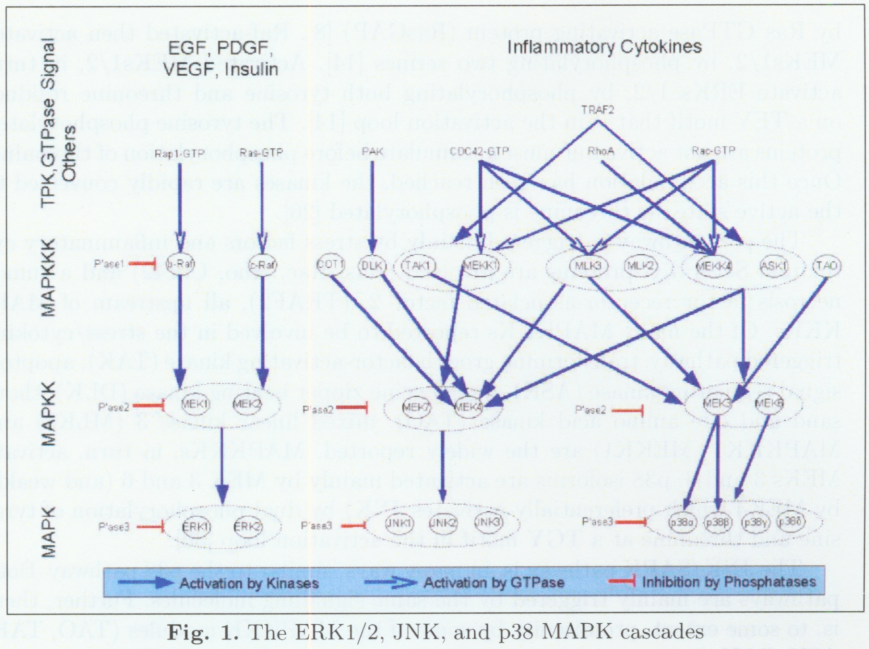


Fig. 1. The ERK1/2, JNK, and p38 MAPK cascades

distinguishable MAPK pathways have been identified so far: extracellular signal-regulated kinases 1 and 2 (ERKs 1/2), c-Jun N-terminal kinases 1,2 and 3 (JNK 1/2/3), p38 ($\alpha/\beta/\gamma/\delta$), ERKs 3/4 and ERK 5 [30]. The most widely studied, in vertebrates, are ERKs 1/2, JNK and p38 [20]. ERKs 1/2, preferentially regulate cell growth and differentiation whilst JNK and p38 are strongly activated by stress and inflammatory cytokines [30, 5]. Although all MAPKs have their own unique properties, they share a number of characteristics. All have a set of three evolutionary conserved kinases: MAPK, MAPKK (MAPK kinase), MAPKKK (MAPK kinase kinase). An activated MAPKKK activates an MAPKK by donating a phosphate molecule. In turn, the MAPKK activates an MAPK downstream of it by phosphorylation, creating a cascade of the involved proteins. The MAPK pathways proceed as shown in Figure 1.[3]

Once a receptor has been activated, in the case of ERKs 1/2, a complex between an adaptor protein Grb-2 and a guanine nucleotide exchange factor SOS interacts with Ras-GDP, activating Ras by exchanging the GDP with GTP. Upon activation, Ras-GTP interacts with Raf (isoforms a,b,c), recruits it to the plasma membrane for subsequent activation. c-Raf and a-Raf are reported to undergo a complex series of activation steps that are not yet fully elucidated whilst it is suggested that the association of Ras-GTP might be sufficient to activate b-Raf [14]. Subsequent deactivating hydrolysis of GTP to GDP is catalysed

[3] The figure can be arrived at through our tool, see Section 5.

by Ras GTPase activating protein (RasGAP) [8]. Raf-activated then activates MEKs1/2, by phosphorylating two serines [14]. Activated MEKs1/2, in turn, activate ERKs 1/2, by phosphorylating both tyrosine and threonine residues on a TEY motif that is in the activation loop [14]. The tyrosine phosphorylated proteins are not active but must accumulate before phosphorylation of threonine. Once this accumulation has been reached, the kinases are rapidly converted to the active state, as threonine is phosphorylated [26].

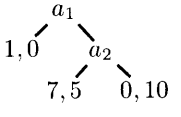
The p38 pathway is triggered mainly by stress factors and inflammatory cytokines. Several G-proteins are involved (Ras, Rac, Rho, Cdc42) and a tumor necrosis factor receptor-associated factor 2, (TRAF2), all upstream of MAPKKKs. Of the many MAPKKKs reported to be involved in the stress/cytokine triggered pathway, transforming growth factor-activating kinase (TAK), apoptosis-signal regulating kinase (ASK), dual leucine zipper bearing kinase (DLK), thousand and one amino acid kinase (TAO), mixed linear kinase 3 (MLK3) and MAPKKK1 (MEKK1) are the widely reported. MAPKKKs, in turn, activate MEKs 3 and 6. p38 isoforms are activated mainly by MEK 3 and 6 (and weakly by MEK4 which preferentially activates JNK) by dual phosphorylation of tyrosine and threonine at a TGY motif in the activation loop [30].

The JNK/SAPK pathway is, in many ways, similar to the p38 pathway. Both pathways are mainly triggered by the same signalling molecules. Further, there is, to some extent, promiscuity by some of the MAPKKK modules (TAO, TAK, ASK, DLK, for example) between the two pathways. Other MAPKKKs involved in the JNK pathway are MLK3, MEKK4 and MEKK1. All phosphorylate substrates MEKs4 and 7 at two serine residues [25]. The activation of JNK isoforms is by dual phosphorylation of a tyrosine and a threonine residue at a TPY motif [30] by MEK 4 and 7. In vitro, MEK 4 preferentially phosphorylates tyrosine while MEK 7 prefers threonine, perhaps suggesting a form of cooperation between these MEKs in the activation of JNK [26].

After activating its downstream effector molecule, each module in the cascade is deactivated by a phosphatase, creating a motif typified by a cascade of cycles. Further, this negative feedback can confer, to individual loops, adaptation and robustness to changes occuring in their environment(s) [12].

3 Game Theory

Non-cooperative game theory is based around the notion of Nash equilibrium. Nash equilibria are defined over *strategies* that account for the intended behaviour of all agents in a game. We say that an agent is *happy* if he cannot change his contribution to a strategy and generate a better overall outcome for himself. A strategy is a Nash equilibrium if all agents are happy with it. An example using a *sequential game* in *extensive form* is as follows, on the left.



	h_1	h_2
v_1	$0, 1$	$1, 0$
v_2	$1, 0$	$0, 1$

A play of the game on the left is a path from the root to a leaf, where the first (second) number indicates the payoff to agent a_1 (a_2). A strategy, by contrast, is a game where a choice has been made in all internal nodes, not just in the nodes on a considered path. While it might look like the strategy of a_1 going right and a_2 going left is good, it is not a Nash equilibrium because a_2 can go right, for a better payoff. At that point, also a_1 can benefit from changing his choice and, in fact, the only Nash equilibrium in the game is a_1 (a_2) going left (right), hence the non-cooperation moniker. Kuhn's Theorem guarantees the existence of Nash equilibria in sequential games in extensive form [16,37]. Kuhn's Theorem is related to the eponymous Nash's Theorem, which addresses the situation of *simultaneous games* in *strategic form* [22,24]. An example is above on the right. In the example, there are two players: vertical, who chooses the row and gets the first payoff, and horizontal, who chooses the column and gets the second payoff. As can be seen, in no outcome are both players happy, i.e., one player always can and wants to move away. This means that it is necessary to consider compromises to guarantee the existence of simultaneous Nash equilibria.

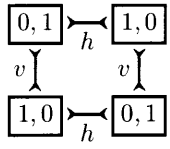
3.1 Rewriting Game Theory

A recent lightweight version of Nash's Theorem due to the corresponding author et al aims to facilitate a wider range of technical applications of game theory, in part by introducing a notion of equilibrium that is discrete and dynamic and in part by removing any and all structural constraints of strategic-form games [31]. The arrived-at notion of *conversion/preference (C/P) game* is intended as the most general structure for which we can define the notion of Nash equilibrium.

Definition 1 (C/P Games [31]) G^{cp} *are 4-tuples* $\langle \mathcal{A}, \mathcal{S}, (\succ_{\mathrm{a}})_{\mathrm{a}\in\mathcal{A}}, (\lhd_{\mathrm{a}})_{\mathrm{a}\in\mathcal{A}} \rangle$*:*

- $\mathcal{A}$ *is a non-empty set of* agents.
- $\mathcal{S}$ *is a non-empty set of* synopses *(read: outcomes of the game).*
- *For* $\mathrm{a} \in \mathcal{A}$, $\succ_{\mathrm{a}}$ *is a binary relation over* $\mathcal{S}$*, associating two synopses if agent* a *can* convert *the first to the second.*
- *For* $\mathrm{a} \in \mathcal{A}$, $\lhd_{\mathrm{a}}$ *is a binary relation over* $\mathcal{S}$*, associating two synopsis if agent* a prefers *the second to the first.*

The idea behind the definition is to make explicit the parts of strategic-form games that are relevant to the definition of Nash equilibria. To illustrate, we note that the example we considered earlier amounts to the following C/P game (with implicit preference relation).



As mentioned, C/P games are set up to facilitate the definition of Nash equilibria in a clean manner.

$$\frac{s \succ_a s' \quad s \lhd_a s'}{s \rightarrow_a s'}$$

Fig. 2. The *(free) change-of-mind* relation for agent a in G^{cp}

Definition 2 ((Abstract) Nash Equilibrium [31]) *Given* G^{cp}.

$$Eq^{aN}_{G^{cp}}(s) \triangleq \forall a \in \mathcal{A}, s' \in \mathcal{S} \,.\, s \succ_a s' \Rightarrow \neg(s \lhd_a s')$$

The first benefit of the more abstract view on simultaneous games that we capture in the C/P game formalism comes from the eponymous fact that conversion and preference facilitates a rewriting characterisation of Nash equilibria.

Definition 3 ([31]) *Given* G^{cp}, *the* change-of-mind *relation,* $\rightarrow_a$, *for agent* a *is given in Figure 2. Let* $\rightarrow \triangleq \bigcup_{a \in \mathcal{A}} \rightarrow_a$.

With this, we see that a Nash equilibrium is a synopsis for which there is no outgoing change-of-mind step, i.e., an $\rightarrow$-irreducible (aka a $\rightarrow$-normal form).

Proposition 4 ([31]) $Eq^{aN}(s) \Leftrightarrow s \in IrR_{\rightarrow}$

The benefits of the changed perspective on game theory are partly conceptual, in the first instance for people that like rewriting, but, secondly, it also captures the informal decision procedure for Nash equilibria that we have employed: a synopsis is a Nash equilibrium if all agents are happy with it, i.e., if no agent wants to move away from it. We account for the technical benefits next.

3.2 Change-of-Mind Equilibria

For our rewriting/graph-theoretic view on game theory, we note that for arbitrary finite graphs only cycles can prevent the existence of terminal nodes. We show in this section how that simple observation suffices for underpinning a discrete version of Nash's Theorem for arbitrary finite C/P games (including strategic-form games). The relevant graph-theoretic notion we need for capturing all cycles is *strongly connected components* (SCC), $\lfloor - \rfloor$, and the *shrunken graph*, $\curvearrowright$, defined over them, see Appendix B.

Theorem 5 ([31]) *For any finite C/P game,* $\langle \mathcal{A}, \mathcal{S}, (\succ_a)_{a \in \mathcal{A}}, (\lhd_a)_{a \in \mathcal{A}} \rangle$,

- $\langle \mathcal{A}, \lfloor \mathcal{S} \rfloor, (\curvearrowright_a)_{a \in \mathcal{A}}, (\curvearrowright_a)_{a \in \mathcal{A}} \rangle$ *has a Nash equilibrium,*[4]
- *all of which can be found in linear time in the size of* $\mathcal{S}$ *and* $\rightarrow$.

Proof The strongly connected components of a finite graph can be found with the stated complexity [35] and so can the shrunken graph and its terminal nodes [21]; the latter are guaranteed to exist in the finite case because a shrunken graph is anti-symmetric (i.e., has no cycles) by construction. □

[4] In Nash's Theorem [22, 24], the strategies are probabilised, rather than SCCed.

Contrary to Nash's Theorem, Theorem 5 does not benefit from instinctive reader recognition of the "shrunken" qualifier of the guaranteed Nash-equilibrium. While Nash's use of probabilities to collect together several strategies/outcomes in his equilibria might instinctively appear better than our approach, we will show shortly that that assessment is not technically justified. In order to make the comparison, we first characterise the Nash equilibria of Theorem 5 directly.

Definition 6 (Change-of-Mind Equilibrium) *Write* $\xrightarrow{S}$ *for* $\rightarrow \cap (S \times S)$.

- $\mathrm{Eq}^{\mathrm{com}}(s) \triangleq \forall s' \in \mathcal{S} . (s \rightarrow^* s' \Rightarrow s' \in \lfloor s \rfloor)$
- $\mathrm{Eq}^{\mathrm{com}}(\xrightarrow{\lfloor s \rfloor}) \triangleq \mathrm{Eq}^{\mathrm{com}}(s)$

We refer to the former notion as change-of-mind equilibrium *points* and the latter simply as change-of-mind equilibria. The concept of change-of-mind equilibrium is well-defined because "membership-in-$\lfloor - \rfloor$" is an equivalence relation and the core result is that they coincide with the Nash equilibria in Theorem 5.

Lemma 7 ([31]) *Consider* $\langle \mathcal{A}, \mathcal{S}, (\succ_a)_{a \in \mathcal{A}}, (\lhd_a)_{a \in \mathcal{A}} \rangle$, $\langle \mathcal{A}, \lfloor \mathcal{S} \rfloor, (\curvearrowright_a)_{a \in \mathcal{A}}, (\curvearrowright_a)_{a \in \mathcal{A}} \rangle$.

$$\mathrm{Eq}^{\mathrm{com}}(\xrightarrow{\lfloor s \rfloor}) \quad \Leftrightarrow \quad \mathrm{Eq}^{\mathrm{aN}}(\lfloor s \rfloor)$$

Change-of-mind equilibria, in other words, are areas where game-play cannot leave, once it has arrived there. Agents are still allowed to change their mind but they remain within a set perimeter (in fact, the smallest such perimeters [31]). More, it is always in some agent's interest to go towards a perimeter/equilibrium.

3.3 Examples

Seen as a C/P game, the strategic-form game at the beginning of Section 3 has one change-of-mind equilibrium.

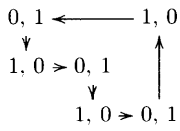
$$\begin{array}{ccc} 0, 1 & \prec & 1, 0 \\ \blacktriangledown & & \blacktriangle \\ 1, 0 & \succ & 0, 1 \end{array}$$

By comparison, we note that it also has one probabilistic Nash equilibrium, arising when both agents choose between their two options with equal probability for expected payoffs of a half to each. In particular, note that the probabilistic Nash equilibrium also uses[5]/compromises between all four outcomes.

The trade-off between Nash's probabilistic approach and our discrete and dynamic approach is that Nash prescribes a definite expected pay-off while we make it clear why the four cells are in the equilibrium. The following two examples illustrate how the two notions differ. Both strategic forms, left and right, have the same change-of-mind equilibrium, centre, when seen as a C/P game.

[5] The technical term 'use' with the given meaning is due to Nash [22, 24].

	h_1	h_2	h_3
v_1	0,1	0,0	1,0
v_2	1,0	0,1	0,0
v_3	0,0	1,0	0,1



	h_1	h_2	h_3
v_1	0, 1	−7,0	1, 0
v_2	1, 0	0, 1	−7,0
v_3	−7,0	1, 0	0, 1
v_4	0, 0	0, 0	0, 0

The only probabilistic Nash equilibrium for the strategic-form game on the left arises when both agents choose between their three options with equal probability, for expected payoffs of a third, using all nine strategies. The change-of-mind equilibrium carves out just six of the strategies, for an *average* payoff of a half to each. In the game on the right, all probabilistic Nash equilibria involve 'vertical' putting full weight on the last row, for expected payoffs of naught to each, using strategies that are disjoint from the six strategies involved in the only change-of-mind equilibrium, centre.

4 Cascaded Protein Games

In this section, we propose a general way of using the C/P game formalism of Section 3.1 to model situations like the one considered in Section 2. Specifically, we will focus on the signalling effect of protein-protein interactions. The methodology will consist of two main steps, described in separate subsections below. We formulate the formal framework using the terminology of signal transduction systems but it is fully algebraic and (in principle) has wider applicability.

Our starting point will be a compendium of chemical data, specifically a list of *catalyst*-indexed *reactions* from *substrates* to *products*. Here is an example.

$$\texttt{ERK} + \texttt{ATP} \xrightarrow{\texttt{MEK-PP}} \texttt{ERK-P} + \texttt{ADP} \tag{1}$$

$$\texttt{ERK-P} + \texttt{H}_2\texttt{O} \xrightarrow{\texttt{Pase}_3} \texttt{ERK} + \texttt{Pi} \tag{2}$$

4.1 Protein Games

As C/P games have few constraints, we can model the listed reactions directly.

Agents Our first step is to extract the set of catalysts from the given set of reactions and consider it as the C/P game's set of agents.

$$\mathcal{A} \quad = \quad \{\texttt{MEK-PP}, \texttt{Pase}_3\}$$

The justification for this is that given a reaction like (1), also the following reaction (without MEK-PP-catalysis) is possible.

$$\texttt{ERK} + \texttt{ATP} \longrightarrow \texttt{ERK-P} + \texttt{ADP} \tag{3}$$

In other words, catalysts do not enable previously unenabled reactions. Instead, they change the affinity for the reaction in question, typically leading to an increase in the rate of reaction by 10^6 to 10^{12} times [38]. It is our thesis that this effect is so significant that narrowly focusing on the control exercised by the enzymes by constructing a C/P game revolving around their "game play" and subjecting that game to an equilibrium analysis will reveal significant information about the expected behaviour of a solution that is accounted for by a considered compendium of chemical reactions.

Synopses The synopses (read: game situations) of the C/P game we are constructing will essentially be defined as a "solution language", encompassing at least all substrates and products in the compendium. Avoiding all issues of an overtly detailed formalism that makes unjustified distinctions between chemical solutions we might not wish to distinguish, we pursue a simplified language. It takes as starting point a classification of the compounds in the compendium as *in-focus* vs *auxiliary* and we simply let the former be the set, $\mathcal{S}$, of synopses. More precisely, we let each in-focus compound be the *characteristic representative* of a synopsis, with all other compounds available as needed. The set of in-focus compounds will typically consist of proteins but it need not be exhaustive.

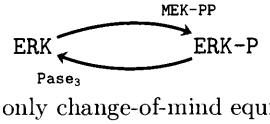
$$\mathcal{S} \quad = \quad \{\texttt{ERK}, \texttt{ERK-P}\}$$

The auxiliary compounds in any given analysis are simply ignored. The notion of characteristic representative is part of the level of abstraction captured by our game-theoretic formalisation. For what it is, namely a first/Ockham-razor approximation, the above "solution language" proposal works remarkably well, see Section 5, but looking into alternatives is naturally part of our future work.

Change-of-Mind In defining the conversion and preference relations, we make the (natural) distinction that conversion is about the chemical reality of the compounds we consider while preference accounts for the catalysing effects of the enzymes. Concretely, we stipulate that there is only one conversion relation shared by all agents and that it comprises reactions like (3). The catalysed reactions will make up the preference relation for that particular catalyst. We would thus typically read (1) to say that MEK-PP prefers (a solution with characteristic protein) ERK-P to (a solution with characteristic protein) ERK. For the example compendium considered at the start of this section, i.e., (1) and (2), the default *protein game* might therefore be accounted for by the following set of agents, set of synopses, and change-of-mind relation.

$$\langle \{\texttt{MEK-PP}, \texttt{Pase}_3\}, \{\texttt{ERK}, \texttt{ERK-P}\}, \{\texttt{ERK} \rightarrow_{\texttt{MEK-PP}} \texttt{ERK-P}, \texttt{ERK-P} \rightarrow_{\texttt{Pase}_3} \texttt{ERK}\} \rangle$$

Presented graphically, it looks as follows.



The whole graph is the only change-of-mind equilibrium of the game.

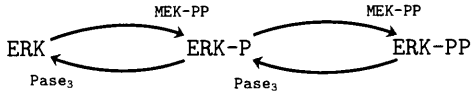
4.2 Composition and Cascading

We will now consider the situation of a chemical compendium consisting of (1) and (2) as well the following two reactions.

$$\texttt{ERK-P} + \texttt{ATP} \xrightarrow{\texttt{MEK-PP}} \texttt{ERK-PP} + \texttt{ADP} \tag{4}$$

$$\texttt{ERK-PP} + \texttt{H}_2\texttt{O} \xrightarrow{\texttt{Pase}_3} \texttt{ERK-P} + \texttt{Pi} \tag{5}$$

As a graphically-presented protein game, the compendium looks as follows, with the two pairs of reactions *composed* by virtue of their overlap on ERK-P.

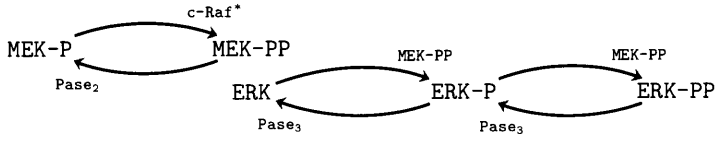


As above, the whole graph is the only change-of-mind equilibrium of the constructed game. We now add two more reactions.

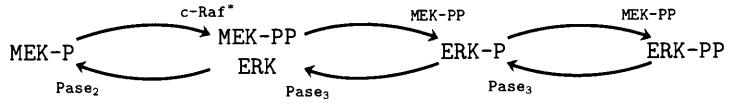
$$\texttt{MEK-P} + \texttt{ATP} \xrightarrow{\texttt{c-Raf}^*} \texttt{MEK-PP} + \texttt{ADP} \tag{6}$$

$$\texttt{MEK-PP} + \texttt{H}_2\texttt{O} \xrightarrow{\texttt{Pase}_2} \texttt{MEK-P} + \texttt{Pi} \tag{7}$$

As a protein game (with overlapping $\mathcal{A}$ and $\mathcal{S}$), the six reactions look as follows.



In the figure, we have two disconnected graphs for which we can observe that the reaction from ERK to ERK-P will be triggered by the production of MEK-PP above it, because MEK-PP is a catalyst for the reaction. This phenomenon occurs regularly and is what is referred to as *cascading*. We will internalise the triggering effect by constructing, for a given protein game, a *cascaded protein game* that collapses, e.g., MEK-PP and ERK.



Technically speaking, we topologically sort the change-of-mind relation of each enzyme and collapse the node of that enzyme with each of the found roots, as exemplified in Figure 5.[6] In our experiments we have only encountered change-of-mind relations that are acyclic — a prerequisite of topological sorting. We cannot imagine what it might mean for an enzyme to catalyse cyclically and we shall therefore not propose an alternative course of action if the topological sort fails. Future work includes more elaborate schemes for implementing cascading.

[6] Note that cascading can occur as one enzyme to many reactions and as many to one.

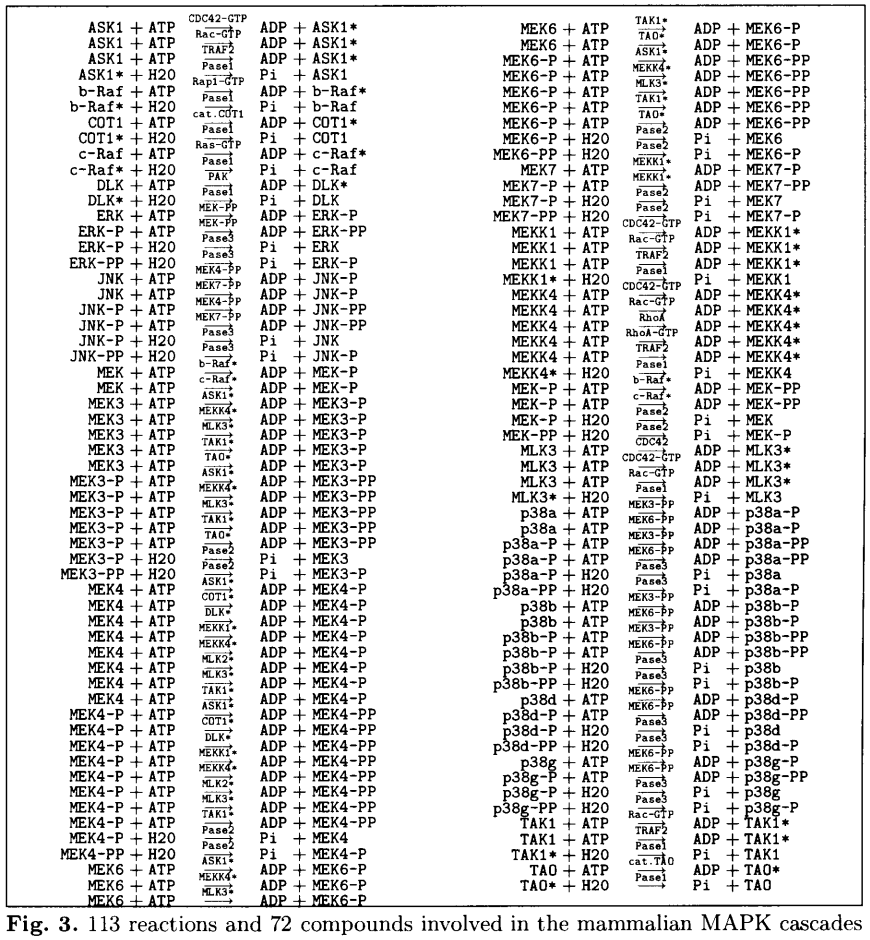


Fig. 3. 113 reactions and 72 compounds involved in the mammalian MAPK cascades

5 A Chemical Compendium and its Equilibria

In this section, we apply our proposed game-theoretic analysis to a medium-sized example, namely all chemical reactions stated in [3, 7, 11, 13, 25, 28, 29, 36] to be involved in MAPK cascades. The articles list a total of 113 distinct reactions, see Figure 3. The reactions involve a total of 72 compounds of which 4 are non-proteins and 14 are proteins that only catalyse. Figure 4 lists the 54 proteins that occur in a substrate or a product. Figures 3 and 4 have been extracted from the content of the variables `MAPKreactions` and `MAPKproteins` used below.

```
MAPKcomEq = findCoMEq[constructCPG[MAPKreactions, MAPKproteins]]
Creating vertices : {2006, 5, 17, 14, 44, 0.1875000}
53 vertices ... {2006, 5, 17, 14, 44, 0.2656250} Done.
```

```
ASK*, ASK1, ASK1*, b-Raf, b-Raf*, COT1, COT1*, c-Raf, c-Raf*, DLK, DLK*, ERK, ERK-P, ERK-PP, JNK,
JNK-P, JNK-PP, MEK, MEK3, MEK3-P, MEK3-PP, MEK4, MEK4-P, MEK4-PP, MEK6, MEK6-P, MEK6-PP, MEK7, MEK7-P,
MEK7-PP, MEKK1, MEKK1*, MEKK4, MEKK4*, MEK-P, MEK-PP, MLK3, MLK3*, p38a, p38a-P, p38a-PP, p38b, p38b-P,
p38b-PP, p38d, p38d-P, p38d-PP, p38g, p38g-P, p38g-PP, TAK1, TAK1*, TAO, TAO*
```

Fig. 4. The 54 proteins occurring in a substrate or a product in Figure 3

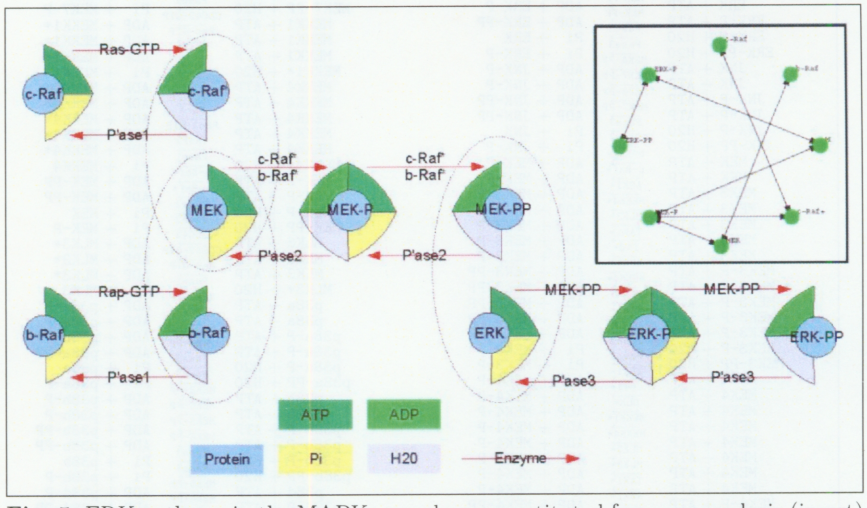


Fig. 5. ERK pathway in the MAPK cascades, reconstituted from our analysis (insert)

```
Creating Change-of-Mind relations : {2006, 5, 17, 14, 44, 0.2656250}
64 (113 enzyme-specified) Change-of-Mind ... {2006, 5, 17, 14, 44, 0.3125000} Done.
Creating collapsed vertices : {2006, 5, 17, 14, 44, 0.3125000}
Find a new graph with Collapse/Duplicate {2006, 5, 17, 14, 44, 0.3125000}
56 collapsed vertices... {2006, 5, 17, 14, 44, 0.3906250} Done.
Creating collapsed Change-of-Mind : {2006, 5, 17, 14, 44, 0.3906250}
128 (291 enzyme-specified) Change-of-Mind ...{2006, 5, 17, 14, 44, 0.4375000} Done.
{2006, 5, 17, 14, 44, 0.4531250} Done.
There are 2 SCCs.
There are 2 non-trivial, and 0 singleton CoM Eq (TSCC).
```

As can be seen, when analysing the reactions in Figure 3 with focus on all reacting proteins, Figure 4, we find 2 change-of-mind equilibria. The second, containing 14 reactions, is reproduced (as output by Mathematica) in the insert in Figure 5. The main part of the figure is a graphical representation of the chemical reactions that lead to the cascaded version seen in the insert. In fact, the computed change-of-mind equilibrium is exactly the classical MAPK cascade: the ERK pathway, i.e., the core part of the transduction system used for signalling cell growth for example, see Figure 1. Seen game-theoretically, this can be read to say that invocation of the ERK pathway is *inevitable*, i.e., it is the best compromise for what the enzymes prefer to do when given a suitable input, cf. Theorem 5. It can also be read more directly to say that the ERK pathway

is a good candidate for a central building block of a living organism because it is *sustainable*, i.e., no enzyme can defect "play" from the pathway once it has arrived there, see Lemma 7.

The second change-of-mind equilibrium found above contains the JNK and p38 pathways and is rather more complex because of the crosstalk that exists between them, see Figure 1. Although we do not discuss it here, we note that our tool allows us to disambiguate the two pathways by focusing away or onto proteins that are particular to one or the other pathway. Similarly, the crosstalk between the two pathways can be analysed by focusing, e.g., on the proteins that sit on direct/no-loop paths between JNK and p38 (and we note that our tool has complementary support for direct-path analysis).

6 Conclusion

We have proposed a lightweight Nash Theorem with discrete and dynamic equilibria as a seemingly suitable starting point for a practically useful tool for the biochemical study of signal transduction systems. The core idea is that the prescribed *change-of-mind* equilibria will amount to likely end-configurations of evolution, i.e., the backbone signalling mechanisms that sustain life (in the ultimate instance). The key concept of the underlying theory is *dynamic equilibria*.

Our proposal comes with preliminary computerised support. We do not consider the proposed technology to have been proven. At best, we believe we have gathered encouraging evidence that is worth pursuing. One issue needing attention are the parameters of our construction of *cascaded protein games*. Another issue is application to raw data that has not been pre-sorted. Theoretically, we also need to extend *rewriting game theory* to accommodate reaction kinetics explicitly. The main advantages of our proposal are conceptual and technical simplicity, low computational complexity (Theorem 5), and perceived generality.

A Abbreviations

K	kinase
ASK	apoptosis-signal regulating K
DLK	dual leucine zipper bearing K
ERK	extracellular signal-regulated K
GCKR	germinal center K receptor
GLK	GCK-like K
GTP	guanosine triphosphate
HGK	HPK/GCK-like K
JNK	c-Jun N-terminal K
MAPK	MAP K
RasGAP	Ras GTPase activating protein
SOS	son of sevenless
TGF-β	transforming growth factor-β
TPY	threonine, proline, tyrosine
ADP	adenosine diPhosphate
ATP	adenosine triphosphate
EGF	epidermal growth factor
GCK	germinal center K
GDP	guanosine diphosphate
Grb2	growth receptor bound proteins
GTPase	GTP phosphatase
HPK1	hematopoetic progenitor K 1
MAP	mitogen-activated protein
MLK	Mixed lineage K
SAPK	stress activated protein K
TAO	thousand and one amino acid K
TGY	threonine, glycine, tyrosine
TEY	threonine, glutamic acid, tyrosine

MEK/MAPKK/MAP2K	MAP kinase kinase
MEKK/MAPKKK/MAP3K	MAP kinase kinase kinase
PTK/TPK	protein tyrosine K/tyrosine protein kinase
TAK	transforming growth factor-activating kinase
TKR/RTK	tyrosine K receptor/receptor tyrosine kinase
TRAF	tumor necrosis factor receptor-associated factor

B Strongly Connected Components, Shrunken Graphs

- A *graph* is a binary relation on a carrier set, called vertices: $\rightarrow \subseteq \mathcal{V} \times \mathcal{V}$.
- The *reflexive, transitive (or pre-order) closure*, $\rightarrow^*$, of a graph, $\rightarrow$, is

$$\frac{v_1 \rightarrow v_2}{v_1 \rightarrow^* v_2} \qquad \frac{}{v \rightarrow^* v} \qquad \frac{v_1 \rightarrow^* v \quad v \rightarrow^* v_2}{v_1 \rightarrow^* v_2}$$

- The strongly connected component (SCC) of a vertex, v, in a graph is

$$\lfloor v \rfloor \triangleq \{v' \mid v \rightarrow^* v' \wedge v' \rightarrow^* v\}$$

  (The relation "is in the $\lfloor - \rfloor$-class of" is an equivalence relation.)
- The set of SCCs of a graph is

$$\lfloor \mathcal{V} \rfloor \triangleq \{\lfloor v \rfloor \mid v \in \mathcal{V}\}$$

- The *shrunken graph* of $\rightarrow \subseteq \mathcal{V} \times \mathcal{V}$ is $\curvearrowright \subseteq \lfloor \mathcal{V} \rfloor \times \lfloor \mathcal{V} \rfloor$, defined by

$$V_a \curvearrowright V_b \triangleq V_a \neq V_b \wedge (\exists v_a \in V_a, v_b \in V_b \,.\, v_a \rightarrow v_b)$$

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